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Variability of soil enzyme activities and vegetation succession following boreal forest surface soil transfer to an artificial hill

R. Maarit Niemi[1], Juha Pöyry[1], Ilse Heiskanen[2], Virva Uotinen[1], Marko Nieminen[3], Kirsti Erkomaa[2], Kaisa Wallenius[1]

1 *Finnish Environment Institute, Natural Environment Centre, P.O. Box 140, FI-00251 Helsinki, Finland* **2** *Finnish Environment Institute, Research and Innovation Laboratory, P.O. Box 140, FI-00251 Helsinki, Finland* **3** *Metapopulation Research Group, Department of Biosciences, P.O. Box 65 (Viikinkaari 1), FI-00014 University of Helsinki, Finland; Faunatica Oy, Lansantie 3 D, FI-02610 Espoo, Finland*

Corresponding author: *Juha Pöyry* (juha.poyry@ymparisto.fi)

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Abstract

A landfill site in southern Finland was converted into urban green space by covering it with a layer of fresh forest humus transferred from nearby construction sites. The aim was to develop the 70 m high artificial hill into a recreational area with high biodiversity of flora and fauna. Forest humus was used as a source of organic matter, plant roots, seeds, soil fauna and microorganisms in order to enable rapid regeneration of diverse vegetation and soil biological functions. In this study we report the results of three years of monitoring of soil enzyme activity and plant species compositional patterns. Monthly soil samples were taken each year between June and September from four sites on the hill and from two standing reference forests using three replicate plots. Activities of 10 different enzymes, soil organic matter (SOM) content, moisture, pH and temperature of the surface layer were monitored. Abundances of vascular plant species were surveyed on the same four hill sites between late May and early September, three times a season in 2004 and 2005. Although the addition of organic soil considerably increased soil enzyme activities (per dw), the activities at the covered hill sites were far lower than in the reference forests. Temporal changes and differences between sites were analysed in more detail per soil organic matter (SOM) in order to reveal differences in the quality of SOM. All the sites had a characteristic enzyme activity pattern and two hill sites showed clear temporal changes. The enzyme activities in uncovered topsoil increased, whereas the activities at the covered Middle site decreased, when compared with other sites at the same time. The different trend

between Middle and North sites in enzyme activities may reflect differences in humus material transferred to these sites, but difference in the succession of vegetation affects enzyme activities strongly. Middle yielded higher β-sitosterol content in 2004, as an indication of more intense plant impact. All reclaimed sites had characteristic plant species assemblages and parallel temporal changes, reflecting vegetation succession, occurred across all the sites. Rapid growth of vegetation on the covered sites restored the rhizosphere and contributed to the persistence of microbial activity. We suggest that transferring the surface soil humus layer is a useful approach for ensuring the outcome of habitat restoration and complementary habitat creation especially in situations where the source soil areas would otherwise be lost.

Keywords
landfill site, mineral soil, soil organic layer, soil enzymes, habitat creation, recreation, vegetation

Introduction

Land and soil use is drastically altered in a growing city, where new territory is needed for housing and for the infrastructure (Pavao-Zuckerman 2008). Novel approaches to enable distribution of soil removed from construction sites on the one hand and to provide green space for recreation on the other, are needed in limited urban space. Furthermore, the question of maintaining sustainable biological processes crucial for providing ecosystem services has recently been recognized important, also within urban areas (e.g. Isbell et al. 2011, Cardinale et al. 2012). Traditionally, surplus soils have been piled, thus mixing the different horizontal soil layers. However, as the biological activity is concentrated in the surface organic layer this procedure weakens soil fertility and recovery of soil functions. Healthy soil is dependent on diverse microbial consortia that have a fundamental role in the decomposition and transformation of organic matter (Kibblewhite et al. 2008). It has been estimated that more than 90% of the energy flow in soil systems passes through microbial decomposers (Nannipieri et al. 2003). The high biodegradation potential of microbial consortia is based on their vast enzymatic capacity. Enzymes involved in the degradation of macromolecules such as cellulose, hemicelluloses, starch and proteins are mainly extracellular and hydrolytic (Burns 1982, Tabatabai and Dick 2002).

Plant root and leaf litter is the primary source of soil organic matter and it affects the quality and quantity of carbon substrates and nutrients available to free-living fungi and bacteria. Helsinki City had constructed an artificial hill using mainly mineral soil removed from construction sites. As a novel approach to the various needs of landscaping, building of recreation areas and restoring biological diversity and functions through utilisation of surface soils removed from discontinued forest sites under constructional development, it was decided to collect the organic surface layer of forest separately and to use this biologically diverse material to cover a barren artificial hill. Fresh forest soil humus was distributed as a layer of a few tens of centimetres on a hill area, excluding steep slopes. The intention was to enhance the development of vegetation on the hill, to improve its recreational value and to restore biological functionality and ecosystem services through the increase in biodiversity (e.g. Forup et al. 2007, Hopwood 2008). However, it must be noted that the interaction of plants and microbes is disturbed

when surface soil is transported to a barren hill constructed from stones and mineral soil, because symbiotic relationships and litter input are heavily affected (Gange and Brown 2002). Vegetation is drastically affected when trees are missing and, in spite of seeds and roots transferred within the humus material, local environmental conditions govern the persistence of transferred plant species and largely determine the trajectory of succession (Gibson and Brown 1991, Mortimer et al. 1998).

Habitat creation is potentially a very efficient tool for enhancing biodiversity in highly disturbed or completely artificial sites. The habitats being created are typically novel ones with plant and animal communities characteristically different from naturally occurring communities (Anderson 1995). Relatively few experimental studies have, however, been implemented on habitat creation in terrestrial environments, even though many projects have been performed to create compensatory habitats (Morris et al. 2006). Nevertheless, factors determining the expected patterns of vegetation succession are not known thoroughly enough to make reliable predictions (Vesk et al. 2008). The available species pool constrains the outcomes that are possible, but also soil type and properties are crucial in the re-vegetation process and largely determine the resulting community (Anderson 1995). Microbiota and soil invertebrate fauna can be regarded as an important soil property affecting vegetation development (De Deyn et al. 2003). Considerably better understanding exists about many aspects of habitat restoration and re-creation (e.g. Anderson 1995, Walker et al. 2004, Hedberg and Kotowski 2010), and most lessons learned in these contexts are also relevant in terms of habitat creation. Perhaps the most fundamental gain for biodiversity conservation, when these novel habitats are created, is the increasing connectivity of habitats and the decreasing risk of regional extinction of species using these habitats. In other words, species or immigration credit is produced (Hanski 2000).

The aim of our study was to monitor temporal changes in the enzyme activity and developing vegetation, and to compare the enzyme activity patterns in surface soil and plant species composition in order to reveal changes due to alteration in vegetation, as well as in litter quality and quantity and in physical conditions important to soil biota (moisture, temperature, pH). Regards to vegetation composition we hypothesize that vegetation succession should in the early phases be faster in moist depression (e.g. Grove) than in more exposed experimental sites (e.g. Top). On enzyme activities we hypothesize that (1) surface soil transfer decreases enzyme activities, (2) increase in vegetation supports enzyme activities in transferred soil and (3) plant species composition affects enzyme activity patterns. The enzyme activity pattern measured consisted of fundamental reactions in macromolecule degradation: arylsulphatase releases inorganic S, phosphomonoestarase and phosphodiesterase release inorganic P from organic molecules, and alanine and leucine aminopeptidases hydrolyze the amino-terminal from amino acids of peptides and some proteins. β-N-Acetyl hexosaminidase degrades chitin. Cellobiohydrolase and β-glucosidase are active in the degradation of cellulose into sugar monomers and β-xylosidase in the hydrolysis of xylo-oligosaccharides produced in the degradation of xylan. α-Glucosidase degrades starch. Soil ergosterol content is widely used as a measure of fungal biomass (Morgan and Winstanley 1997,

Wallander et al. 1997). Because fungi in soil are important producers of extracellular enzymes and are probably affected by forest soil transfer, when ectomycorrhiza are no longer supported by trees, we also measured ergosterol content. β-Sitosterol, which reflects plant impact on soil, including litter, was also assayed (Sinsabaugh et al. 1997). Monitoring the changes in plant species composition are in wide-spread use in the studies of successional changes on reclaimed habitats and were also applied here to describe the general successional patterns on the experimental areas.

Methods

Study area and sampling

The study area is a former landfill site situated in Helsinki City, in the proximity of the Gulf of Finland. A total of about $5*10^6$ m^3 of mineral soil and non-biodegradable construction waste was transferred to the artificial hill site area of 38 hectares between 1990 and 2002. The organic surface layer of forest soil was distributed to the hill sites, excluding steep slopes and the hill top, in spring 2003. Heavy machinery (typical to mine industry and construction) was used for the collection, transportation and distribution on the soil cover. Due to the large-scale operation, characteristics of the humus material collected over large forest areas and level of mixing with mineral soil may have varied. Soil cover depth varied also, when organic material was spread over an uneven terrain. Middle and North sites were covered with surface soil from clear cut spruce forest and Grove was covered with clay and surface soil from an alder grove. Later Grove was planted with siblings of ash (*Fraxinus excelsior*) and hazelnut (*Corylus avellana*) from the site of the organic surface soil.

Three replicate 10 m × 10 m plots of four hill sites (Middle, North, Grove and Top) and of two reference sites were selected for soil microbial activity studies (Fig. 1, Table 1). Growing forests in the proximity of the sites of origin of the transported surface soils were used as reference sites (Alder for Grove and Spruce for Middle and North). Photographs of the studied sites at the onset of the study in June 2003, in August 2003, in August 2005 and after a decade in June 2013 are presented in electronic Supplementary Figures S1–S4. Soil samples were taken once a month from June to September each year from 2003 to 2005 as composite surface sample cores (depth 5 cm, diameter 3.4 cm) of 20 random subsamples from each plot. Samples were transferred to the laboratory refrigerated and were stored at +5 °C overnight. The next day, samples were passed through a 4 mm sieve.

Vegetation survey

For studies of vegetation composition, the abundance of all vascular plant species was surveyed in the same hill site (Middle, North, Grove and Top) replicate plots as

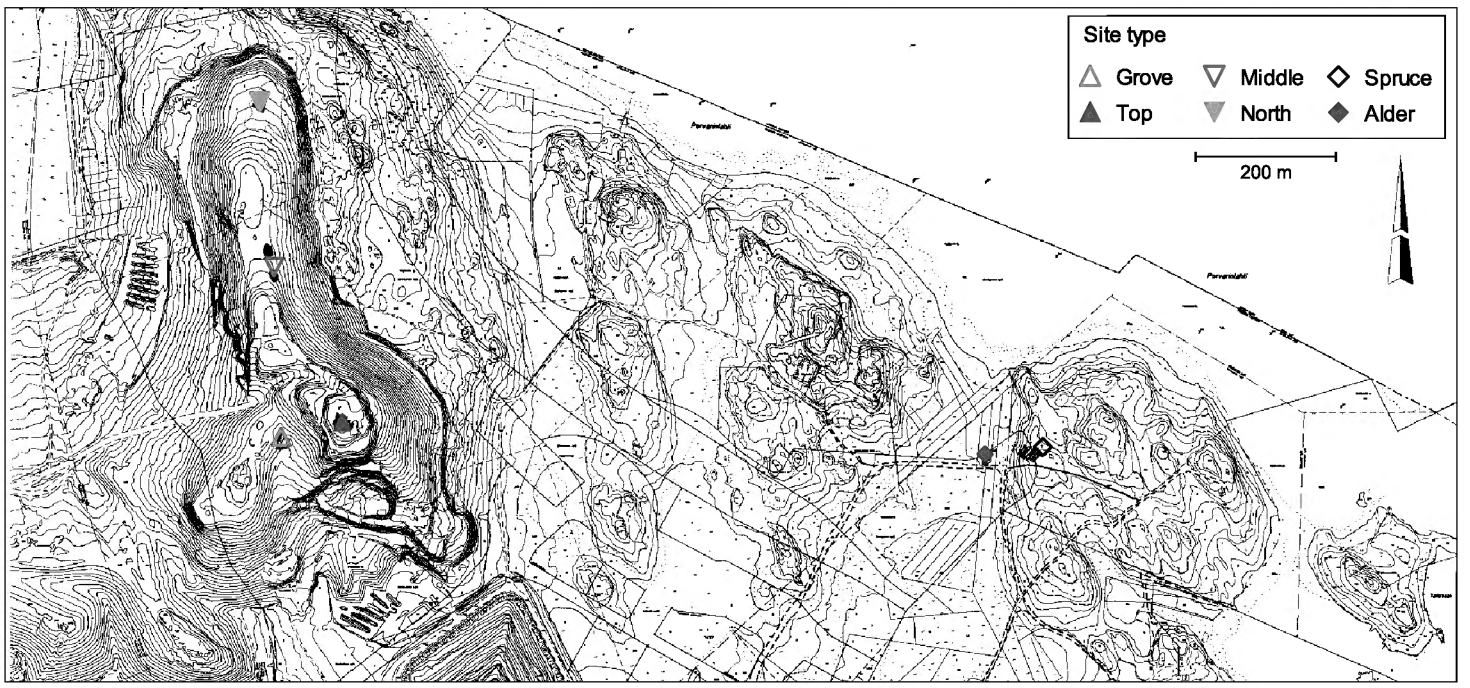

Figure 1. Map of the study area. The study area is situated in Helsinki City (60°13'N, 25°10'E).

Table 1. Characteristics of the sites monitored (see Fig. 1).

Site	Characteristics
Top	The top of the hill consisted of moraine. Vegetation was sparse but increased slightly during the monitoring.
Grove	Indentation on the southwest side of the hill. The bottom was covered with clay to prevent water infiltration and the clay layer was covered with surface soil from a black alder (*Alnus glutinosa*)-dominated grove in spring 2003. These two layers were not well separated and the surface soil contained variable patches of clay. Sparse vegetation of plants typical to the grove was observed during the first summer, but later the vegetation of different species dominated, e.g. very dense *Cirsium* growth.
Middle	Ridge site in the middle of the hill. Sandy moraine was covered with surface layer of old *Picea abies*-dominated forest. Vegetation was sparse during the first summer. Later *Agrostis* (2005), *Carex* (2004), *Luzula* (2004), *Poa* (2004) and *Rubus* (2004) species were common.
North	Ridge site in the north side of the hill. Sandy moraine was covered with surface layer of old *Picea abies*-dominated forest. Vegetation was sparse during the first summer. Succession later yielded very dense *Deschampsia* growth and in 2004 *Betula* (cut) and *Luzula* were common.
Alder	Old *Alnus glutinosa*-dominated brookside grove on the sea shore.
Spruce	About 60 years old *Picea abies*-dominated forest.

in the microbial activity study three times (late May, early July and early September) in 2004 and 2005. Plant abundance was estimated using a frequency method in which the number of positive records across sampling plots is summed and used to describe the commonness of a species. Here we applied this method so that each replicate plot was further divided into nine subplots and the number of subplots with positive records of a species was then used as the measure of abundance of vascular plants in plots (Greig-Smith 1983). Plants were identified according to Hämet-Ahti et al. (1998). We acknowledge here that the experiment was confined to a single landscape thus lacking spatial replications, and therefore generalization of the results to other areas should be cautionary. Subsamples taken from one treatment area

(Middle, North, Grove and Top) were combined before applying statistical tests to avoid pseudoreplication that may arise if spatial dependence of sampling is not accounted for (cf. Hurlbert 1984). However, all subsamples are shown in ordinations for enzyme and vegetation data (Fig. 3 and Fig. 5) to explore the total variation occurring in these data.

Physical and chemical measurements

The sieved samples were stored at +5 °C for 1 to 7 d before measuring dry weight, loss on ignition and pH_{KCl} in duplicate. For the measurement of soil dry weight and water content, fresh samples were dried at 105 °C overnight. Soil organic matter content (SOM) was determined by loss on ignition at 550 °C. For the pH measurement, 10 g of soil was weighed to 50 ml of 1 mol l^{-1} KCl solution in a screw cap bottle. After 10 min shaking at 200 rpm and settling for 2 h, pH was measured from the liquid phase using an Orion 550A electrode. Soil moisture and temperature were measured in the field at 5 cm depth on all the sampling dates from each plot at 20 random points using an HH2 Moisture Meter- instrument with a WET-sensor (Delta-T Devices Ltd). The means of 60 measurements for each site were calculated.

Ergosterol and β-sitosterol measurements

Samples were stored at +5 °C for 3 d and then, depending on the SOM content, 0.9 to 5.5 g aliquots were weighed into distillation flasks and 50 ml methanol (Rathburn, HPLC quality) was added and the suspensions were stored well capped at –20 °C until analysed for ergosterol and β-sitosterol. The slightly modified method of Sinsabaugh et al. (1997) was used: The total volume of methanol was made up to 100 ml before refluxing the samples for two hours. For saponification, 5 ml of 4% KOH in ethanol was added and the samples were refluxed for an additional 30 min. The undigested material was removed by filtration through glass wool. After addition of 10 ml of water into the methanol solutions the samples were extracted with three 10 ml portions of n-pentane (Merck) in separation funnels. The pentane of the samples was evaporated in a rotavapor (Büchi) and the solid residues were reconstituted in 1 ml of methanol before measuring the sterol contents by HPLC (Hewlett-Packard Model 1090 equipped with diode arrow detector and an analytical column of Hypersil ODS 200x2.1 mm with 5 µm particle size). Ergosterol was detected at 280 nm and β-sitosterol at 205 nm. Retention time for ergosterol was 6.9 min and for β-sitosterol 10.5 min when 50% methanol-water was used as an eluent with a flow rate of 0.4 ml/min. Quantitation was based on comparison of peak heights of sterols in the sample and in the standard solutions. A calibration curve was established for ergosterol (Fluka, purum 98%) from 0.6 to 46.5 µg/ml and for β-sitosterol (Sigma, S 9889, 98.3%) from 3.7 to 283.5 µg/ml.

Enzyme activity measurements

Enzyme activities were measured from 4 g samples stored in small plastic bags at –20 °C for 6 to 38 d (Wallenius et al. 2010) using ZymProfiler test kits (Vepsäläinen et al. 2004, Vepsäläinen et al. 2001). We measured the activities of arylsulphatase (no data for 2005), α-glucosidase (α-Glu), β-glucosidase (β-Glu), β-xylosidase (β-Xyl), cellobiohydrolase (Cell), β-N-acetyl hexosaminidase (Chi), phoshodiesterase (PDE), phosphomonoesterase (PME), and alanine- (AlaAP) and leucine aminopeptidases (LeuAP). Homogenized samples were suspended in 0.5 M acetate buffer at pH 5.5 and 1:100 dilutions (or 1:1000 dilutions for PME activities) were pipetted into multiwell plates containing pre-dried fluorogenic artificial substrates and incubated with shaking for 3 h at 30 °C. The fluorescence was measured with a Victor^2 multilabel analyzer (Perkin-Elmer) from four replicate wells on the plates. For the standardization, the curves of eight different concentrations of methylumbellipherone and for the aminopeptidases aminomethyl coumarine, in three replicates, were measured for each sample and dilution.

Statistical analyses

Multivariate ordination methods with non-metric multidimensional scaling (NMDS; see (Clarke 1993, McCune and Grace 2002) as implemented in the software PC-ORD, version 5.33 (McCune and Mefford 2006) were used to explore the main patterns of variation in enzyme activities and plant species composition between the experimental sites and their subplots. Quantitative version of the Sørensen (i.e. Bray-Curtis) distance measure was used to calculate the site-to-site dissimilarity matrix used in ordination, and varimax rotation was used in order to enhance correlation between the main component of variation and the first ordination axis (McCune and Grace 2002). The NMDS 'scree plots' were inspected to select the final number of axis dimensions included in the NMDS run (McCune and Mefford 2006).

We performed two NMDS runs with both the enzyme activity and plant species data, one with all the replicate samples handled separately and the other using monthly site means in the analysis but using otherwise similar settings. After performing the first NMDS run, Pearson correlations between axis scores for sites and environmental variables were calculated. Next, multiresponse permutation procedures (MRPP), a method designed for testing group-wise differences (Zimmerman et al. 1985), was applied as implemented in the software PC-ORD, version 5.33 to test whether sites belonging to different experimental treatments differed in ordination space. Quantitative version of the Sørensen (i.e. Bray-Curtis) index was used as distance measure in MRPP (McCune and Mefford 2006). Results from the second NMDS run were used to draw an ordination plot with successional vectors illustrating temporal changes in soil enzyme activity and plant species composition. Plant abundance measures were logarithmically transformed in the first NMDS in order to obtain a more stable solution.

Cluster analysis using Gower's coefficient and Ward's method was applied for enzyme activity data calculated per loss on ignition (SOM) using home tailored programs (ZymProfiler).

Results

Physical and chemical characteristics

The surface soils of the reference forests, with rather even terrain and trees providing shadow, were more moist, contained more organic matter and had lower temperatures than the hill sites (Table 2). Alder soil was not as acid as Spruce soil. Top site not covered by forest soil was driest, contained least organic matter of the hill sites and was less acid than forests and hill sites covered with forest soil. Grove was first covered with clay layer and then with surface soil from black alder (*Alnus glutinosa*)-dominated deciduous forest and the impact of clay is reflected as low organic matter content and moisture on the one hand, but as the highest pH of all the sites on the other. The two sites covered with spruce forest surface layer, Middle and North, were different: More mineral soil was mixed with the surface organic layer of spruce forest transported to North than to Middle and this was seen as lower moisture and organic matter content and higher pH than in Middle. The summer of 2003 was very warm in July, and especially surface soil temperatures on the hill were high.

Ergosterol and β-sitosterol contents

The samples were analyzed for sterols at the end of July and September in 2004 (Table 3). Both sterols had highest concentrations in soil of the reference forests, relatively high concentrations in Middle and less in North but low concentrations in Grove and Top, when calculated per soil dw. When calculated per SOM, the content of ergosterol was highest in the soil of reference forests, second highest in Middle and North, less in Grove and least in Top. The content of β-sitosterol was highest in the soil of reference forests and Middle, less in North and least in Grove and Top Pearson correlations between β-sitosterol and ergosterol were higher per dw ($r=0.84$, $n=33$) than per SOM ($r=0.62$), indicating differences in SOM quality. β-Sitosterol per dw correlated strongly ($r=0.96$, $n=33$) and ergosterol per dw also strongly ($r=0.90$, $n=33$) with SOM.

Enzyme activity and vegetation patterns

Enzyme activities per dry soil were clearly highest in the reference forest and lowest in Top not covered with forest soil organic layer (Fig. 2). Middle yielded higher activities

Table 2. Ranges and medians for each site and year for the physical and chemical characteristics.

		Water content %		SOM %		Temperature		pH_{KCl}	
Site	**Year**	**Range**	**Median**	**Range**	**Median**	**Range**	**Median**	**Range**	**Median**
Spruce	2003	33–48	43	37–46	40	10 -25	14	3.2	3.2
	2004	36–58	50	31–40	38	11–18	17	3.3–3.7	3.5
	2005	36–49	38	38–41	39	13–19	15	3.6–3.7	3.7
Alder	2003	39–54	43	34–39	37	10–24	14	3.8–4.0	3.8
	2004	51–64	58	39–47	42	11–18	16	4.1–4.3	4.2
	2005	45–61	52	41–49	44	14–20	15	4.3–4.6	4.4
Middle	2003	23–32	30	18–20	19	9.6–29	14	3.4–3.6	3.5
	2004	25–42	35	18–26	22	9.7–22	19	3.5–3.7	3.6
	2005	25–37	26	24–25	25	12–22	15	3.6–3.7	3.6
North	2003	11–20	16	6.5–7.9	7.0	11–30	15	3.9–4.0	3.9
	2004	10–25	19	7.0–12	7.4	11–23	19	3.9–4.1	4.0
	2005	12–20	16	6.4–8.0	7.4	12–19	15	4.1–4.2	4.1
Grove	2003	13–22	19	4.4–8.5	6.7	9.1–30	15	5.3–5.6	5.4
	2004	19–29	23	3.8–7.0	5.6	10–26	22	5.4–5.6	5.5
	2005	20–27	21	5.7–7.2	6.4	14–23	15	5.6–5.7	5.7
Top	2003	4.2–13	10	1.3–1.5	1.4	9.5–30	15	4.7–4.8	4.7
	2004	5.4–15	13	1.5–1.8	1.6	10–25	22	4.6–4.8	4.8
	2005	7.1–12	8.7	1.3–1.8	1.6	14–23	16	4.7–4.8	4.8

than North and Grove. PME activity was the highest in all the sites but the enzyme activity pattern was site-dependent. Middle had repeatedly the highest enzyme activities on the hill, clearly higher than North, which had lower SOM content and moisture but was less acid. Enzyme activities normalised by SOM content were used to reveal more sensitively the differences between sites and the possible temporal changes within SOM.

A two-dimensional solution was achieved with NMDS ordination for the enzyme activity data calculated per SOM (final value of the stress function = 9.50). A joint plot of the ordination for experimental sites is presented in Fig. 3. Axis 1 represented 87.7% of the variation of distance measures in the original data set, and axis 2 represented 8.0% of the variation. According to an MRPP analysis, the experimental treatments were separated in the ordination space ($p < 0.001$). Furthermore, pair-wise comparisons by MRPP showed that all treatment groups were differently ($p < 0.001$) distributed in the ordination space, with Top and Middle treatments being the most distinct and Spruce and Alder treatments showing some resemblance (Fig. 3). The vectors of the three most important environmental variables, SOM (loi), dry weight and soil pH (pH_{KCl}), were correlated to axis 2 so that SOM showed a positive correlation ($r = 0.62$) and dry weight and soil pH (pH_{KCl} negative correlations ($r = -0.58$ and -0.53, respectively). This observation indicates that these environmental variables formed a gradient parallel to axis 2 that correlated with the observed variation in enzyme activity patterns.

Table 3. Ergosterol and β-sitosterol (μg/g) in soil in 2004. Standard deviations in parentheses.

		Per dw		Per SOM	
Site	**Date**	**Ergosterol**	**β-sitosterol**	**Ergosterol**	**β-sitosterol**
Spruce	July 27th	38 (±7)	139 (±27)	127 (±26)	468 (±102)
	Sept 27th	75 (±22)	236 (±74)	199 (±52)	605 (±84)
Alder	July 27th	37 (±14)	203 (±159)	99 (±12)	472 (±149)
	Sept 27th	49 (±32)	275 (± 222)	100 (±37)	518 (±183)
Middle	July 27th	14 (±7)	138 (±95)	54 (±6)	487 (±96)
	Sept 27th	15 (±8)	130 (±76)	68 (±5)	551 (±140)
North	July 27th	3.6 (±1.0)	20 (±6)	48 (±5)	262 (±38)
	Sept 27th	4.0 (±2.2)	27 (±10)	56 (±26)	382 (±98)
Grove	July 27th	1.6 (±0.5)	8.4 (±1.2)	30 (±5)	163 (±22)
	Sept 27th	1.4 (±0.3)	6.1 (±2.3)	38 (±10)	162 (±68)
Top	Sept 27th	0.3 (±0.2)	3.0 (±3.0)	18 (±5)	145 (±89)

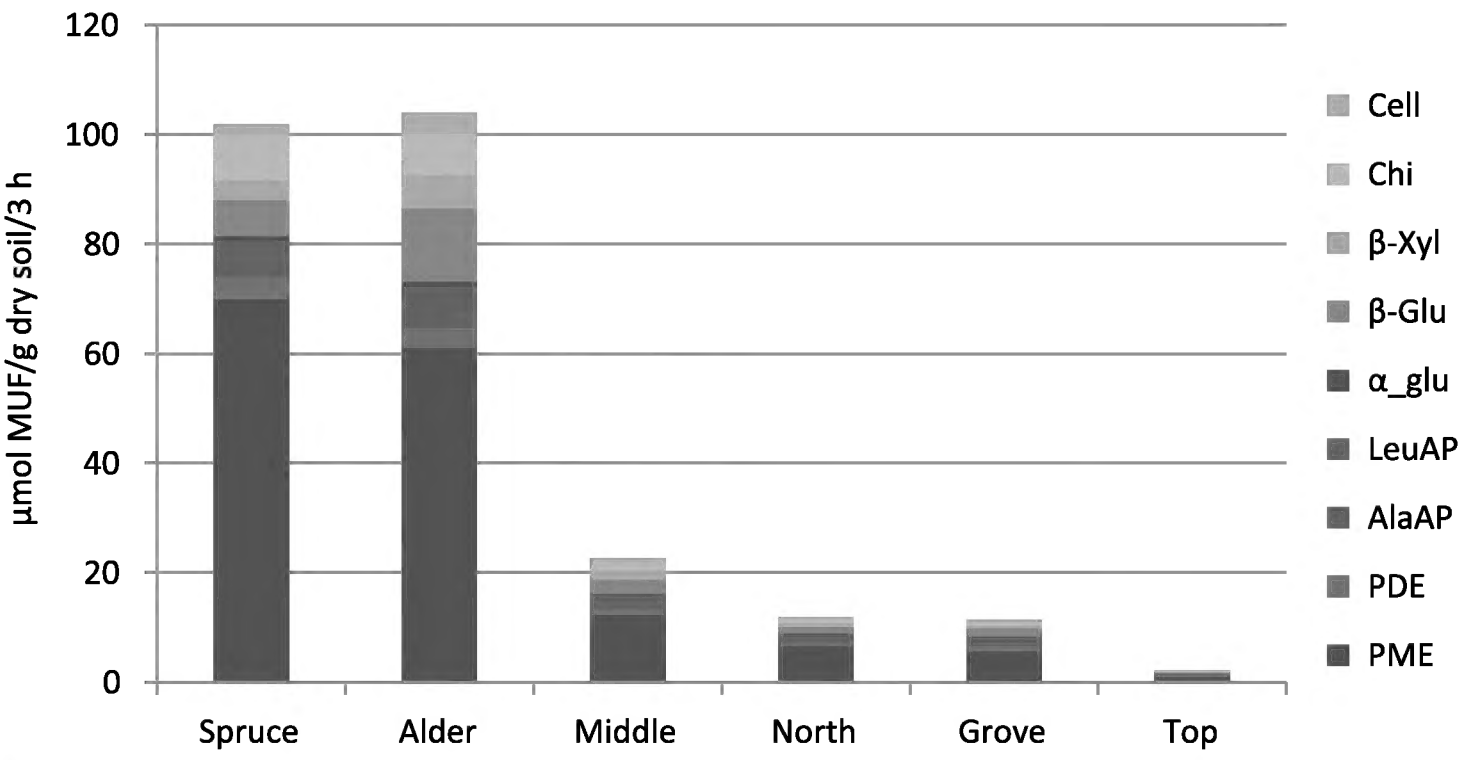

Figure 2. Sums of different enzyme activities in different sites per soil. Medians in three replicate plots calculated per dw from June to September in 2003, 2004 and 2005.

A two-dimensional solution was also achieved with NMDS ordination including monthly means of within-site replicate plots of enzyme activities (final value of the stress function = 8.75). Successional vectors joining monthly samples of different sites (i.e. experimental treatments) showed directional changes in two sites, with Top site moving closer to Grove and North and Middle gradually diverging from the other areas (Fig. 4).

A three-dimensional solution was achieved with NMDS ordination for the plant species composition data (final value of the stress function = 10.52). A joint plot of the ordination for experimental sites is presented in Fig. 5. Axis 1 represented 19.4% of the variation of distance measures in the original data set, axis 2 represented 29.8%

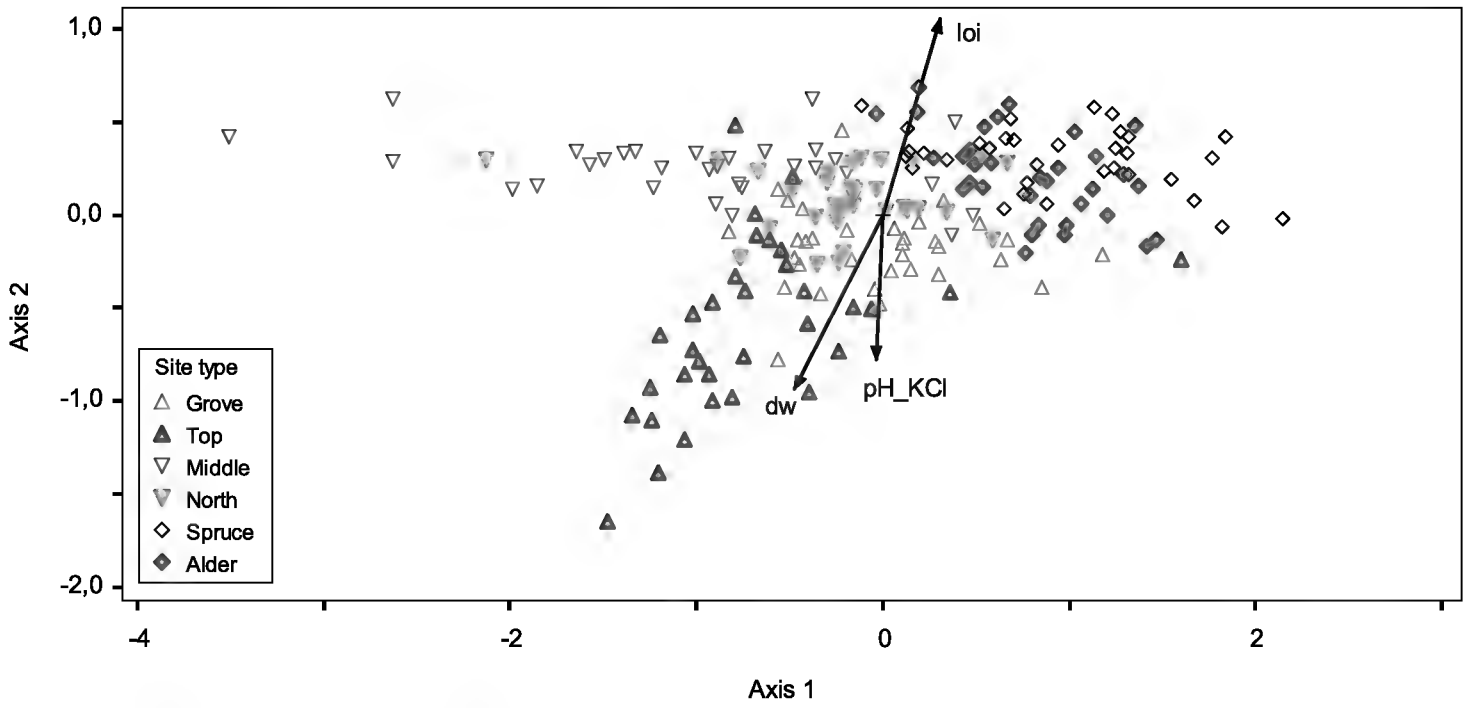

Figure 3. The joint plot of NMDS ordination for enzyme activity per SOM. All replicate plots of each study site with samples taken in June, July, August and September in 2003, 2004 and 2005. The vectors of environmental variables with strongest correlation to ordination axes ($r > |0.5|$) are shown.

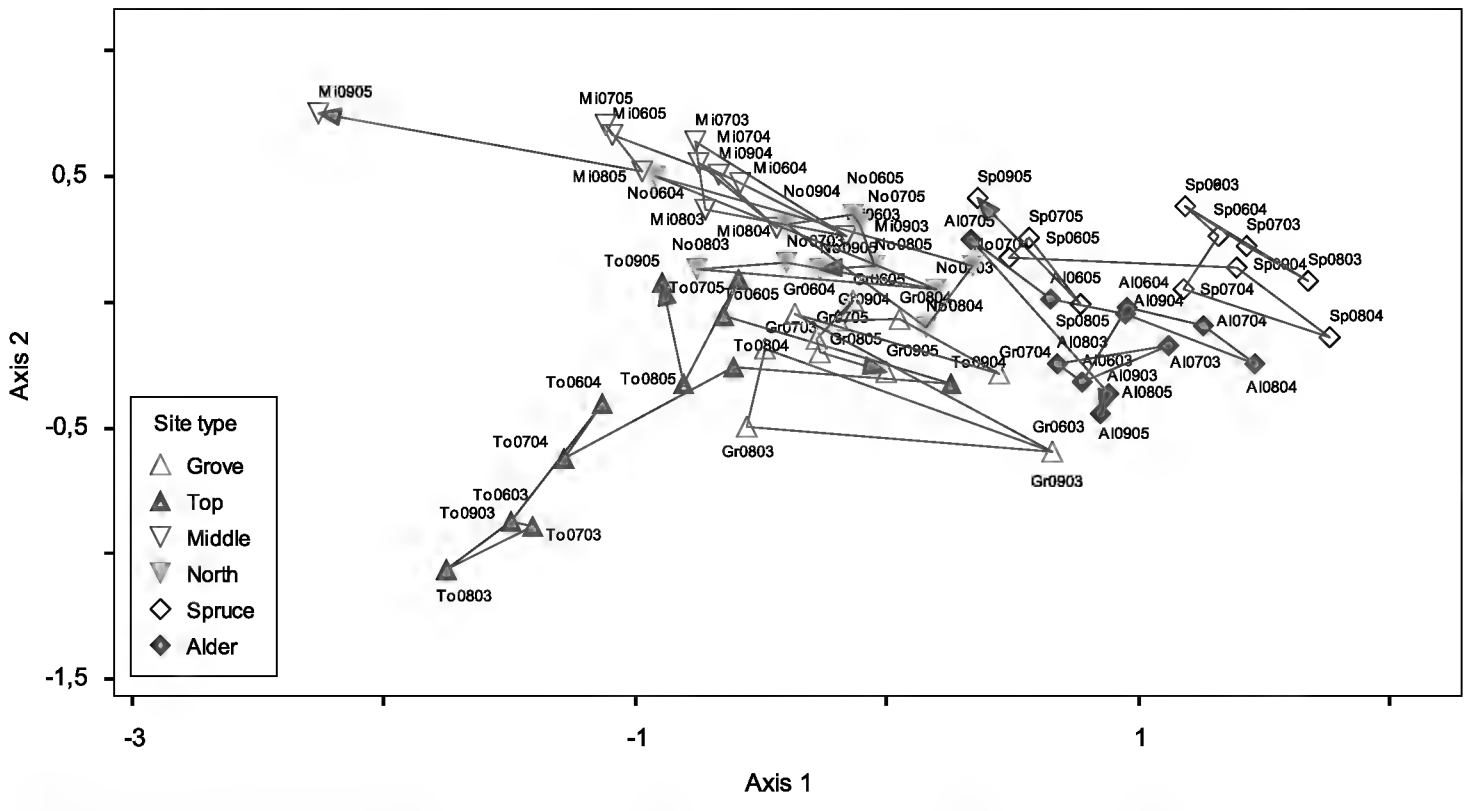

Figure 4. The NMDS plot showing temporal change in enzyme activity per SOM. Successional vectors joining the monthly means of replicate samples of each study site (i.e. experimental treatment) taken in June, July, August and September in 2003, 2004 and 2005. Point labels show two initials of the site, sampling month and year (e.g. Gr0603 = Grove June 2003).

and axis 3 represented 32.9% of the variation. According to an MRPP analysis, the experimental treatments were separated in the ordination space ($p < 0.001$), and pair-wise comparisons by MRPP showed that all treatment groups were differently ($p < 0.001$) distributed in the ordination space in years 2004 and 2005, with Top and

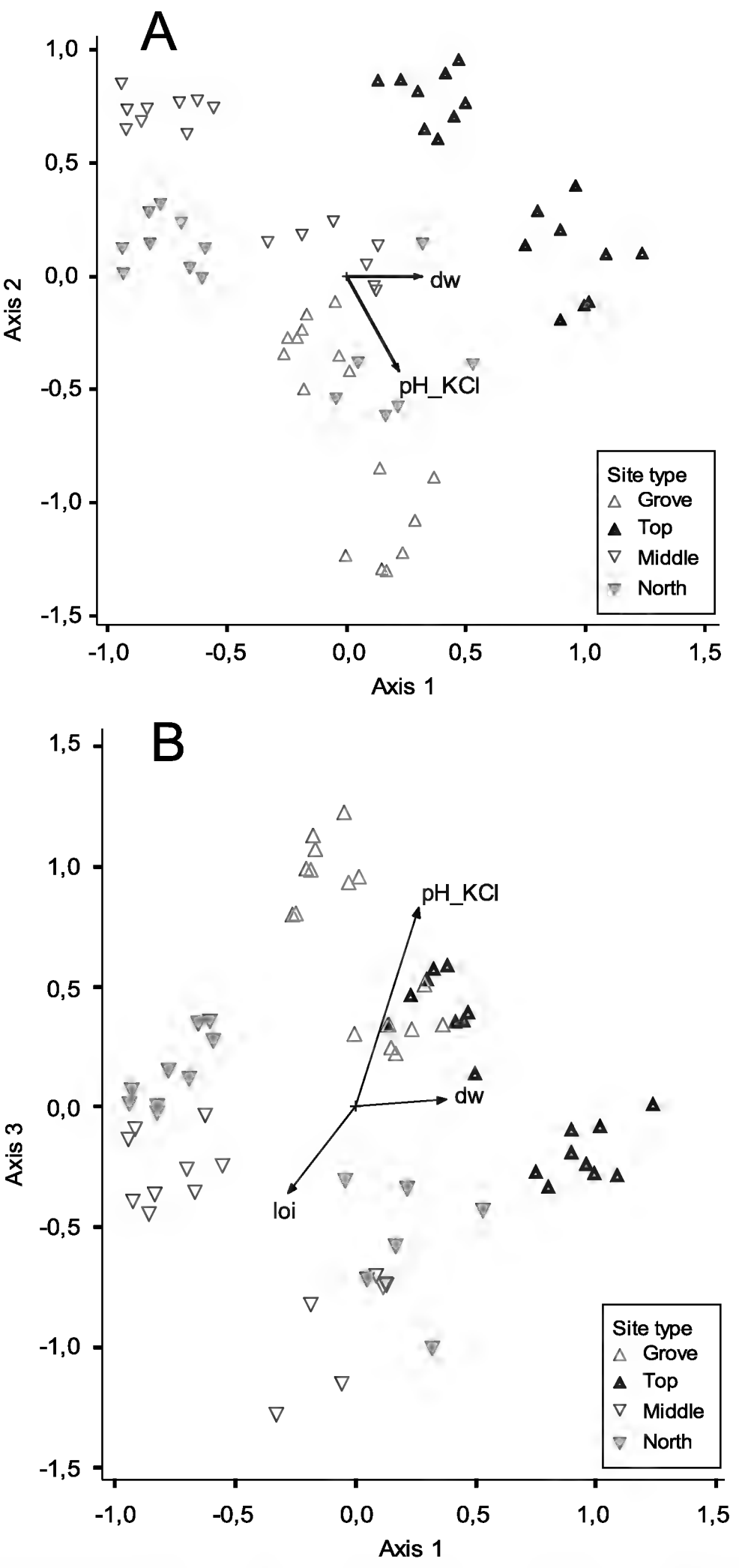

Figure 5. The joint plot of NMDS ordination for plant species composition. All replicate plots of each study site with vegetation surveys done in late May, July and early September in 2004 and 2005. The vectors of environmental variables with strongest correlation to ordination axes ($r > |0.5|$) are shown. The upper panel (**a**) includes axis 1 and axis 2 and the lower panel (**b**) includes axis 1 and axis 3.

Grove treatments now being the most distinct (Fig. 5). The vectors of the two environmental variables, soil dry weight (dw) and soil pH (pH_{KCl}), were positively correlated to axis 1 and axis 3 (r = 0.50 and 0.74, respectively) whereas soil pH was additionally negatively correlated with axis 2 (r = -0.58). This observation indicates that these environmental variables did not form a readily observable gradient as in the enzyme activity ordination.

A three-dimensional solution was also achieved with NMDS ordination including monthly means of within-site replicate plots of plant composition data (final value of the stress function = 5.09). Successional vectors joining monthly samples of experimental treatments showed parallel directional changes in all four areas (Fig. 6). The 20 most abundant plant species were widely scattered around Grove, Middle and North samples on the ordination plots (Fig. 6). There is a tendency for species of more nutrient-rich and moist environments (e.g. *Cirsium arvense* and *Filipendula ulmaria*) to group close to Grove samples, but no strong relationships are evident. However, no species grouped close to Top samples, which probably reflects the sporadic vegetation due to harsh environmental conditions there.

The cluster analysis on the basis of enzyme activities was applied separately for the data for the years 2003 and 2005. The pattern was characteristic to each site at the onset of the study in 2003 and, with one exception, all the samples from different dates formed a site specific sub-cluster (Fig. 7). The reference forests, Middle + North and Grove + Top formed the main clusters. During the next year the activity patterns changed only slightly (data not shown), but they changed further in 2005 and a different picture was seen (Fig. 8). Site-specific groups were again observed, but the autumn samples from Alder reference forest were different (higher activities were generally observed) from the other forest samples. North + Grove formed a main cluster. Top was closer to this cluster, whereas Middle was most different from the other groups.

The relative enzyme activities per SOM between sites from 2003 to 2005 (Figs 7 and 8) changed and variation was observed for the reference forests. PME, chitinase, β-xylosidase and β-glucosidase activities decreased in Middle, β-xylosidase decreased in Grove and chitinase increased in Top from 2003 to 2005. Arylsulphatase, not included in the cluster analysis due to lacking data for 2005, displayed the highest activities calculated per SOM in Grove and very high activities were observed in September 2004 both in Grove and in Top sites (data not shown).

Discussion

Differences between sites

β-Sitosterol present in plant membranes indicates phytobiomass in soil and it has been shown that in early stages of decomposition it is degraded at about the same rate as bulk litter (Sinsabaugh et al. 1997). The strong correlation between β-sitosterol and SOM reflects the high proportion of relatively fresh litter in SOM in the study sites.

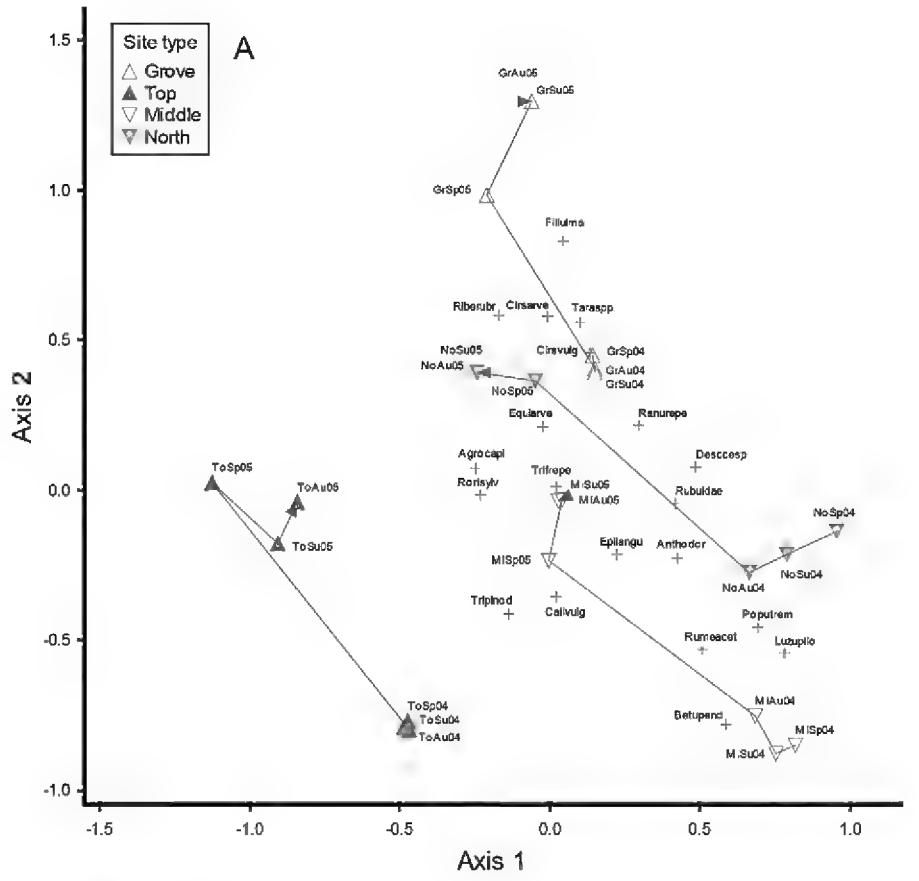

Figure 6. The NMDS plot showing temporal change in plant species composition. Successional vectors joining the monthly means of replicate surveys of each study site (i.e. experimental treatment) done in late May, July and early September in 2004 and 2005. The upper panel (**a**) includes axis 1 and axis 2 and the lower panel (**b**) includes axis 1 and axis 3. Point labels show two initials of the site, sampling season and year (e.g. GrSp04 = Grove Spring 2003). Other season abbreviations are: Su = summer and Au = Autumn. Average positions in the ordination space are depicted for the 20 commonest species according to vegetation surveys. Species abbreviations show four initials of the genus and species names, respectively (see Table S1).

Sample	PME	PDE	LeuAP	AlaAP	Chi	α-Glu	ß-Xyl	Cell	ß-Glu
North07	1.91	0.92	0.76	0.87	0.97	0.49	0.93	0.51	1.19
North09	1.96	0.92	0.76	0.87	**1.17**	0.50	0.97	0.55	1.12
North06	2.06	0.94	0.77	0.89	1.10	**0.54**	1.01	0.67	1.19
North08	*1.86*	*0.82*	0.80	0.92	0.90	**0.56**	0.90	0.50	*1.03*
Grove07	1.90	0.95	0.86	1.00	*0.70*	0.46	*0.73*	0.60	1.21
Middle06	1.97	*0.77*	*0.71*	*0.81*	0.95	0.40	0.89	*0.43*	1.14
Middle09	1.98	*0.71*	*0.70*	*0.83*	0.85	0.36	0.94	*0.38*	1.05
Middle08	*1.83*	*0.66*	*0.62*	*0.77*	0.94	0.34	0.85	*0.45*	1.10
Middle07	1.86	*0.56*	*0.57*	*0.68*	*0.70*	*0.21*	*0.75*	*0.29*	*0.92*
Alder08	2.18	*0.81*	0.93	1.04	1.08	0.26	**1.04**	**0.74**	**1.39**
Alder09	2.16	0.84	0.82	0.96	**1.15**	0.28	**1.10**	**0.72**	**1.30**
Alder07	**2.27**	0.84	0.87	0.98	**1.23**	0.36	**1.09**	**0.87**	**1.45**
Alder06	**2.19**	1.05	0.81	0.97	1.05	0.38	**1.19**	**1.00**	**1.56**
Spruce06	**2.28**	0.85	*0.69*	*0.81*	1.09	*0.22*	0.88	*0.42*	1.10
Spruce09	**2.28**	0.90	*0.72*	*0.77*	**1.24**	*0.21*	0.86	0.52	1.05
Spruce07	**2.33**	1.00	0.75	0.85	**1.36**	0.28	0.95	0.59	1.25
Spruce08	**2.39**	1.01	0.83	0.94	**1.29**	0.36	**1.02**	0.68	1.28
Grove08	*1.85*	**1.15**	0.99	**1.11**	1.06	**0.59**	0.89	0.71	**1.34**
Grove09	2.05	**1.11**	**1.08**	**1.20**	0.91	**0.75**	0.85	**0.75**	1.26
Grove06	2.14	**1.14**	**1.00**	**1.20**	1.14	**0.69**	**1.05**	**0.90**	**1.49**
Top06	*1.64*	1.05	**1.01**	**1.11**	*0.65*	*0.22*	*0.73*	0.61	*0.57*
Top08	*1.55*	**1.09**	**0.99**	**1.13**	*0.66*	*0.12*	*0.71*	0.45	*0.69*
Top07	*1.67*	**1.11**	**1.04**	1.10	*0.72*	**0.56**	*0.76*	0.59	*0.71*
Top09	1.87	**1.07**	**1.05**	**1.15**	*0.63*	0.54	*0.71*	*-0.03*	*0.78*

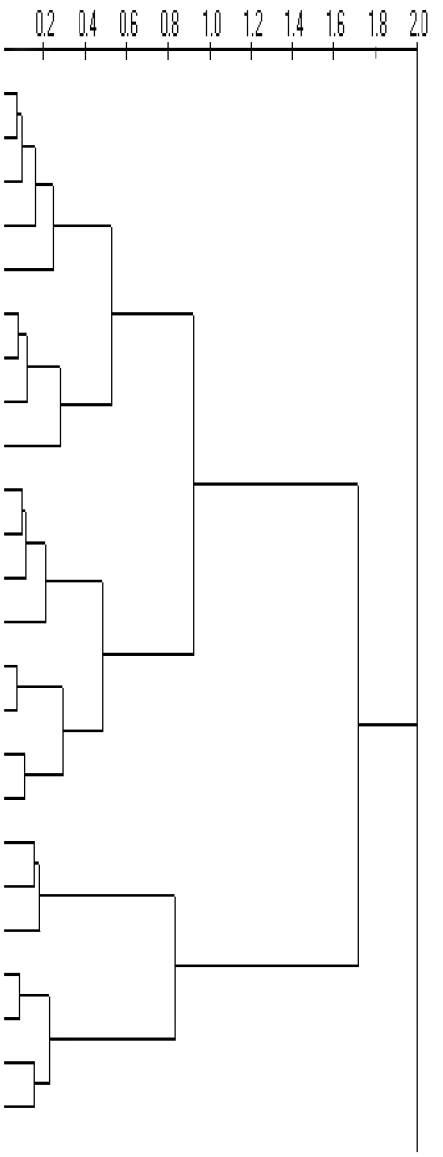

Figure 7. The dendrogram obtained by using means of enzyme activities per SOM in 2003. Triplicate samples of each site in June–September, lg transformed data (µmol MUF or AMC/g SOM in 3 h), standardised data, Gower's similarity coefficient and Ward's method. The original enzyme activity data is arranged according to the dendrogram to reveal differences between clusters. Sampling month (mm) is given for each sample. For each activity, the lower quartile is shown in *italics* and the upper quartile in **bold**.

There was also strong correlation between SOM and ergosterol, the indicator of fungal biomass, which shows fungal occurrence either as saprophytes but possibly also associated with new plant species developed on the constructed sites. Middle contained more β-sitosterol than North per SOM but due to the higher SOM content in Middle, the concentrations were reversed per dw.

Enzyme activity levels in soil per dw were clearly different between sites, true forests yielding the highest activities, followed by sites covered with old coniferous forest organic layer, of which Middle displayed higher activities than North, still lower activities in a site covered with alder forest soil with less organic matter and the lowest activities in hill top covered with mineral soil. The measurement of catabolic respiration patterns (Stevenson et al. 2004) reflect compositions of active microbiota in soil. Our results on soil enzyme activity patterns, affected by substrate availability and stabilisation of enzymes on soil surfaces in addition to the composition of microbiota,

Sample	PME	PDE	LeuAP	AlaAP	Chi	α-Glu	ß-Xyl	Cell	ß-Glu
Alder08	**2.14**	1.06	**1.08**	1.07	**1.39**	**0.51**	**1.27**	**1.05**	**1.61**
Alder09	**2.12**	1.03	**1.07**	**1.14**	**1.43**	**0.52**	**1.29**	**1.09**	**1.63**
Alder06	**2.11**	1.01	0.90	0.98	1.25	0.37	**1.21**	**0.94**	**1.45**
Alder07	2.04	*0.94*	0.88	0.94	1.23	0.14	**1.11**	**0.86**	1.40
Spruce06	**2.10**	1.08	0.87	*0.89*	**1.26**	0.24	0.99	0.75	1.28
Spruce07	**2.11**	1.10	*0.82*	*0.84*	**1.35**	0.32	*0.95*	0.69	1.24
Spruce09	2.08	*0.96*	0.89	*0.92*	**1.26**	0.15	*0.82*	0.51	1.09
Spruce08	**2.16**	1.11	1.05	1.12	**1.45**	0.22	1.02	0.67	1.31
Top06	1.86	**1.45**	**1.10**	**1.14**	1.01	0.21	1.11	0.77	1.09
Top09	*1.80*	**1.42**	**1.07**	1.07	*0.97*	0.18	1.10	*0.45*	*1.07*
Top07	1.90	**1.41**	1.02	1.10	1.01	*0.10*	1.09	*0.03*	*1.03*
Top08	*1.82*	**1.41**	**1.20**	**1.24**	1.07	*0.10*	**1.13**	0.73	*0.92*
North06	1.96	1.10	*0.81*	0.93	*0.95*	0.44	1.01	0.64	1.26
North07	1.95	1.19	*0.82*	0.97	1.03	0.36	1.07	0.73	1.28
North08	1.97	1.17	0.96	1.09	1.07	0.39	**1.12**	0.61	1.30
North09	1.90	1.08	1.01	1.01	1.10	**0.46**	1.04	0.68	1.37
Grove06	1.94	1.24	0.96	1.08	1.02	**0.56**	1.01	0.84	**1.44**
Grove09	1.96	**1.29**	1.00	**1.14**	1.17	**0.63**	1.06	0.84	**1.53**
Grove08	1.90	1.20	**1.10**	**1.23**	1.04	*0.14*	1.03	**0.93**	1.41
Grove07	1.90	**1.26**	0.97	**1.12**	1.01	*-0.97*	1.01	**0.86**	**1.46**
Middle06	*1.77*	*0.80*	*0.68*	*0.77*	*0.76*	*0.33*	*0.93*	*0.45*	*1.09*
Middle07	*1.78*	*0.79*	*0.66*	*0.74*	*0.77*	*0.08*	*0.85*	*0.37*	*1.07*
Middle08	*1.76*	*0.81*	0.85	0.93	*1.00*	**0.48**	*0.92*	*0.46*	*1.07*
Middle09	*1.53*	*0.79*	*0.57*	*0.68*	*0.65*	*0.12*	*0.70*	*0.11*	*0.92*

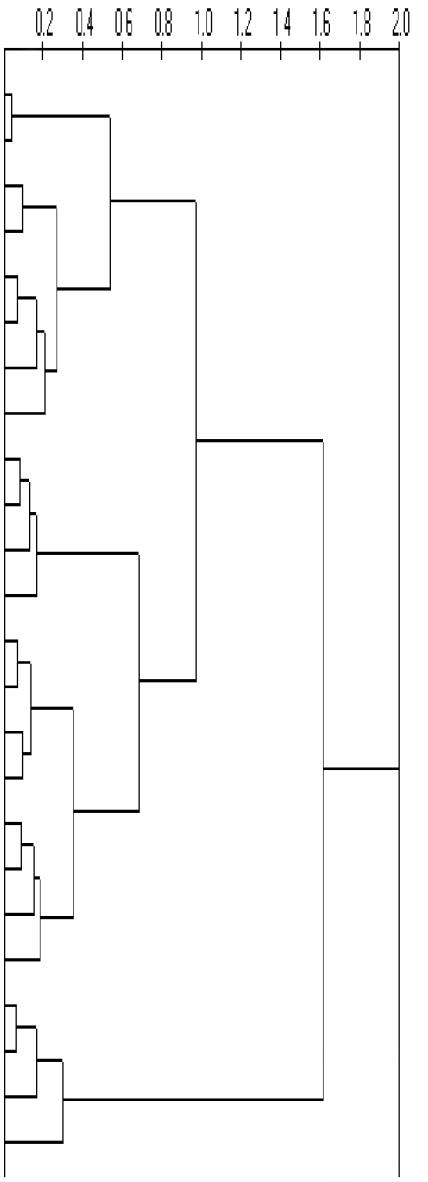

Figure 8. The dendrogram obtained by using means of enzyme activities per SOM in 2005. Explanations as in Fig. 7.

are in agreement with the observation by Stevenson et al. concerning the sensitive differentiation of soils with varying land use.

NMDS ordination revealed that the different study sites had characteristic enzyme activity and plant species compositional patterns. The enzyme activity patterns, normalized by calculation per SOM, were clearly dependent on pH, SOM content and mineral matter content (loi revealing organic matter and dw reflecting mineral contents). This observation is in accordance with the results of previous studies (Niemi et al. 2007, Niemi et al. 2008, Štursová and Baldrian 2011, Wallenius et al. 2011). Surface soil moisture and temperature were less important, possibly due to seasonality simultaneously affecting all the sites and the fact that enzyme activity measurements were carried out under constant conditions and not at *in situ* moisture, pH and temperature. Furthermore, the actual enzyme activities in soil environment depend on substrate availability, whereas activity measurements were carried out without substrate limitation. The enzyme activity results reflect mainly the content of each active enzyme at the time of sampling, which is dependent on the composition of microbiota (Zimmermann et al. 2007). The vegetation compositional patterns were also characteristic for the experimental treatments, apparently illustrating differences in soil seed banks among

the source areas from where surface soil was obtained and transferred to the treatment areas. This observation emphasizes the importance of soil seed bank in determining the plant species composition during the initial stages of vegetation succession that was already evident in the two years (2004–05) following the onset of the experiment (Supp. Figs S1–S3).

The study sites were clearly different in pH but each hill site exhibited a rather stable pH. The importance of pH for enzyme activity patterns plausibly reflected compositions of microbial consortia (Niemi and Vepsäläinen 2005). High SOM content tends to bring about low pH in coniferous soil due to organic acids, whereas high mineral and clay content tend to increase pH. The most important factors affecting enzyme activity patterns are interdependent.

In accordance with Niemi and Vepsäläinen (2005), cluster analysis revealed that relatively high mineral content and pH were associated with elevated PDE and aminopeptidase activities (Top and Grove). On the other hand, high SOM content and low pH were associated with high PME and chitinase activities (Spruce). High ergosterol content in Spruce plausibly indicates the importance of mycorrhizal fungi for these activities. Elevated activities of these enzymes and those responsible for cellulose and hemicellulose biodegradation, namely cellobiohydrolase, β-glucosidase and β-xylosidase, were evident even in Alder with relatively high pH. In general, many enzyme activities normalised by SOM content were the highest in true forests with mycorrhizal symbionts, diverse vegetation and high litter input. Our results conform with results on successional gradient of native prairie restoration showing that changes in microbial biomass were largely attributable to changes in SOM and N concentration together with root biomass (Allison et al. 2007).

Temporal change

One of the main aims was to study how enzyme activities persist in soil transferred from forest to the open hill. Activities calculated per dry soil (Fig. 2) were generally clearly lower in soils on the hill than in the forests at the onset of the study. This depended on the SOM content, which was much lower in the hill sites due to mixing with mineral soil. However, no decreases were observed during the monitoring. It is plausible that plant growth secured the rhizosphere effect and microbial activity even if the vegetation changed. The differences and changes within SOM were analysed in detail.

Both NMDS ordination and cluster analysis revealed a temporal successional change in enzyme activity patterns calculated per SOM between years. The hill top consisting primarily of mineral soil became more fertile, and in photographs taken in 2013, ten years after starting the experiment this change is also reflected by increasing cover of vegetation (Supp. Figs S1–S4). Unfortunately we don't have data on enzyme activities for this later time period, and thus quantitative comparisons with the early successional phase could not be performed. During the first three years of monitoring (2003-2005) after the onset of the experiment some enzyme activities were observed to increase also in Top soil SOM. The change in SOM quality might have been due

to emerging vegetation and very slight increase in SOM content (14% but hardly statistically significant). Middle and North developed differently. North surface layer contained more mineral soil from the onset of the experiment, which kept it dryer and with higher pH, and also yielded lower sterol contents per dry soil. Its SOM contained the same level of ergosterol, indicating similar fungal biomass, and less β-sitosterol, indicating a less strong plant impact, than Middle. More dense grass (Poaceae), especially *Deschampsia flexuosa*, vegetation developed in Middle than in North (Supp. Table S1). However, enzyme activities increased in North, when compared with Middle, revealing different development in rhizosphere and impact on microbial activity. Dense grass cover reduces or even outcompetes many other low-growing plants which is detrimental to biodiversity, because the number of herbivorous insects and their associate species declines severely (e.g. Morris 2000, Pöyry et al. 2006). The relative change between North and Middle was mainly caused by increase in many enzyme activities in North rather than by decreases in Middle.

NMDS ordination also showed changes in plant species composition that occurred in all study areas towards the same direction during the first two years of monitoring that covered early phases of the vegetation succession. These changes clearly indicate the early phases of plant succession (Supp. Table S1, Figs S1–S3), following the establishment of plant species initially from the seed bank that was preserved in the transferred surface soil. Vegetation succession was fastest in Middle and North, whereas in both the moist depression (Grove) and the most exposed site (Top) change was slower based on the axis 1 of NMDS ordinations (no apparent differences in temporal change among sites on axes 2 and 3; see Figs 5 and 6). This pattern was against what we hypothesized. It may reflect a larger species pool being able to grow in more average environmental conditions of Middle and North sites than in more extreme conditions of Grove and Top sites. Therefore, various factors such as interspecific competition, antagonistic relationships and stochastic processes operating with more species can create faster and bigger changes.

The exact reason that would explain the parallel directional change across all experimental treatments remains unclear as none of the explanatory variables measured from the soil samples was correlated with the observed changes in plant composition, but one potential explanation is the increasing vegetation height caused by the same dominant species during early succession. Unfortunately, as with enzyme activities, we do not have data for plant occurrences for the period of ten years after the onset of the experiment (Fig. S4), and thus quantitative comparisons between the early phases of vegetation succession and the later time period were not possible. Grove site has developed to a true grove, but North and Middle sites still look like pastures after a decade.

Conclusions

A landfill site covered with forest soil top layer developed rapidly to urban green space. Soil enzyme activities increased markedly due to the forest soil cover, but remained lower

than in the true forests. Soil enzyme patterns were characteristic to each site. They changed during a 3 year monitoring period reflecting differences in developing vegetation.

The value of integration of soil ecological knowledge with other successional patterns, such as changes in vegetation, in restoration management has been emphasised by several authors (Anderson 1995, Heneghan et al. 2008, Vauramo and Setälä 2010). Our study confirms the advisability of this approach. The transfer of forest organic surface soil to the mineral soil hill clearly increased soil biological activity for the three year duration of the monitoring. Microbial activity level followed the SOM content but the quality of the SOM affected the enzyme activity patterns. Vegetation increased rapidly in the sites covered with forest organic soil and even the establishment of mycorrhizal symbiosis is expected to be enhanced in covered areas (Lunt and Hedger 2003). We suggest that transferring the surface soil humus layer is a useful approach for ensuring the outcome of habitat restoration and complementary habitat creation especially in situations where the source soil areas would otherwise be lost.

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Supplementary material 1

Table S1

Authors: Maarit Niemi, Juha Pöyry, Ilse Heiskanen, Virva Uotinen, Marko Nieminen, Kirsti Erkomaa, Kaisa Wallenius

Data type: occurrence data

Explanation note: Vegetation data used in the ordination analyses.

Supplementary material 2

Figure S1

Authors: Maarit Niemi, Juha Pöyry, Ilse Heiskanen, Virva Uotinen, Marko Nieminen, Kirsti Erkomaa, Kaisa Wallenius

Data type: photograph

Explanation note: The studied sites at the onset of the study in June 2003: **a**) Top **b**) Grove **c**) Middle **d**) North **e**) Alder **f**) Spruce.

Supplementary material 3

Figure S2

Authors: Maarit Niemi, Juha Pöyry, Ilse Heiskanen, Virva Uotinen, Marko Nieminen, Kirsti Erkomaa, Kaisa Wallenius

Data type: photograph

Explanation note: The studied sites in August 2003: **a**) Top **b**) Grove **c**) Middle **d**) North.

Supplementary material 4

Figure S3

Authors: Maarit Niemi, Juha Pöyry, Ilse Heiskanen, Virva Uotinen, Marko Nieminen, Kirsti Erkomaa, Kaisa Wallenius

Data type: photograph

Explanation note: The studied **s**ites in August 2005: **a**) Top **b**) Grove **c**) Middle **d**) North.

Supplementary material 5

Figure S4

Authors: Maarit Niemi, Juha Pöyry, Ilse Heiskanen, Virva Uotinen, Marko Nieminen, Kirsti Erkomaa, Kaisa Wallenius

Data type: photograph

Explanation note: The studied **s**ites after a decade in June 2013: **a**) Top, **b**) Grove **c**) Middle **d**) North.